Potential of Miana Leaves (*Coleus scutellarioides* (L.) Benth) As an Antibacterial *Streptococcus Pneumonia, Staphylococcus Aureus, Staphylococcus Epidermidis, Klebsiella Pneumonia* from Sputum Cough Patients in Makassar City

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Abstract

**Preliminary.** The high intensity of a cough attack is an indication of a respiratory disorder or infection in the throat. Phlegm in coughing indicates infection and inflammation of the respiratory tract. Cough-causing bacteria include *Streptococcus pneumonia, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus epidermidis.* **The aim** of the study was to prove the potential of Miana leaves as an antibacterial cause of cough in vitro. **Method.** The test bacteria *Streptococcus pneumonia, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus epidermidis* are the result of isolation and identification of cough sufferers sputum in Ibnu Sina Hospital Makassar. Sputum samples were taken randomly for a period of 2 weeks. Miana leaves are obtained from Tana Toraja Regency and extracted using a juicer. Antibacterial antibacterial testing was carried out by agar diffusion method. Blank paper disc was soaked in miana leaf extract, cefadroxil, amoxicillin and distilled water as negative control, then placed on the surface of the media containing test bacteria. The inhibition zone is measured as an antibacterial activity. **Results.** Miana leaf extract can inhibit and kill the growth of *Streptococcus pneumonia bacteria, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia.* Miana leaf extract provides a better zone of bacterial growth inhibition than amoxicillin and cefradoxyl. Diameter of inhibition zone of miana leaf extract 25.3 - 28.7mm; cefadroxil 16.6 - 21mm and amoxicillin 11.6 - 20mm. **Conclusion.** Miana leaf extract has the potential as an antibacterial for *Streptococcus pneumonia, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia* which causes coughing.

**Key words:** Miana leaves, Infectious bacteria, Sputum, Cough

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PRELIMINARY

Coughing is one of the body’s main immune mechanisms to cleanse the throat and respiratory tract from dangerous antigenic substances (Baratawijaya, 2010). The high intensity of a cough attack is an indication of a disturbance or infection in the throat. Phlegm in coughing indicates infection and inflammation of the respiratory tract (Ringel, 2009). Cough is classified as a productive cough and non-productive cough. Productive cough is a cough that produces phlegm (Wilson, 2005). Some diseases that are manifested by symptoms of phlegm cough are pneumonia, chronic obstructive pulmonary disease (COPD) acute exacerbations, acute bronchitis, bronchial asthma and bronchiectasis (Danusantoso, 2000). According to the WHO report that every year 4 out of 13 million deaths in developing countries are caused by respiratory infections.

The most common pathogens detected with sputum culture are bacteria such as Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, and Klebsiella species (Ibrahim, 2012). Altiner et al. (2009) found bacteria in acute cough sputum namely Streptococcus pneumonia, Haemophilus influenza, Haemophilus parainfluenza and Moraxella catarrhalis. Parhusip (2004) at BP4 Medan found that the most bacteria causing lower respiratory tract infections were Streptococcus viridians and Staphylococcus aureus. Research by Ziyade and Yagci (2010) found that bacteria from sputum culture with lower airway sufferers were Haemophilus influenza, Pseudomoas aeruginosa and Streptococcus pneumoniae. Panggalo et al. (2013) found bacteria in sputum culture from patients with phlegm cough in Manado Hospital were Streptococcus, Staphylococcus aureus, Proteus, Enterobacter aerogenes, Acinetobacterbaumannii, Seratia marcescens, Hafnia alvei, citrobacter diversus, E coli and Klebsiella ozaenae. Pakadang et al. (2017) found bacteria from sputum culture of cough sufferers in Ibnu Sina Makassar Hospital namely Streptococcus pneumonia, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus epidermidis and Enterobacter agglomerans and Candida albicans fungi.

Treatment of phlegm cough can be done with expectorant drugs and antibacterial from chemicals. As an alternative treatment can also use herbal ingredients that are antibacterial, expectorant and immunostimulant. One of the plants that has been used by the community as a material for the prevention and treatment of cough disease, especially in Toraja in Tana Toraja Regency, South Sulawesi, is Miana. Besides being empirically used, Miana leaves have also been widely studied as a prevention and treatment of diseases. Although miana leaves have
been empirically proven as cough medicines, research needs to be done for scientific evidence. This study aims to determine the potential of Miana leaves as an antibacterial cause of phlegm cough in vitro.

**MATERIAL AND METHOD**

**Preparation**

Miana leaf extract was obtained from Tana Toraja Regency, South Sulawesi province. Miana leaf extract test material was prepared using a juicer. Amoxicillin and cefadroxyl are diluted at 50 ppm. Sputum cough sufferers are taken randomly in a one-week period from Ibnu Sina Hospital Makassar. Selected samples were 10 non-tuberculosis sputums which were isolated and identified in the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University. The test bacterial suspension was prepared with turbidity levels equivalent to Mc Farland 0.5.

**Antibacterial testing**

In vitro testing using agar diffusion method with Mueller Hinton Agar (MHA) media. Blank paper discs soaked in the test material were then placed on the surface of the media containing test bacteria. The suspension is spread evenly using a sterile swab on the media surface of the MHA. Incubate in an incubator at 37°C for 24-30 hours and show the results of the growth inhibition zone around the paper disk.

**RESULT**

Testing the antibacterial potential of Miana leaf extract in vitro with the diffusion method to give results according to table 1.

<table>
<thead>
<tr>
<th>The test bacterial</th>
<th>Replication</th>
<th>Inhibition zone of bacterial growth after being treated with test material (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Miana leaf</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>1</td>
<td>28</td>
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<td></td>
<td>2</td>
<td>28</td>
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<td></td>
<td>3</td>
<td>30</td>
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<td>Average</td>
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<td>28,7</td>
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<td>Klebsiella pneumonia</td>
<td>1</td>
<td>28</td>
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<td></td>
<td>2</td>
<td>25</td>
</tr>
<tr>
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<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>26</td>
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<td>1</td>
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<td>28</td>
</tr>
</tbody>
</table>

**Table 1.** Test results for the potential of miana leaf extract on the inhibition zones of growth of the bacteria *Streptococcus pneumonia, Klebsiella pneumonia, Staphylococcus aureus and Staphylococcus epidermidis*
### The test bacterial Replication | Inhibition zone of bacterial growth after being treated with test material (mm) | Miana leaf | Cefadroxyl | Amoxicillin | Sterile water
--- | --- | --- | --- | --- | ---
*Staphylococcus aureus* | 2 | 25 | 21 | 19 | 0
| 3 | 26 | 20 | 20 | 0 | 
| Average | **26.3** | **21** | **19.6** | **0** | 
| *Staphylococcus epidermidis* | 1 | 25 | 16 | 16 | 0
| 2 | 27 | 16 | 17 | 0 | 
| 3 | 24 | 18 | 17 | 0 | 
| Average | **25.3** | **16.6** | **16.6** | **0** | 

### DISCUSSION

This study used Miana leaf extract to prove the potential of Miana leaves as a scientific cough medicine. Miana is one of the types of plants belonging to the local wisdom of the people of South Sulawesi, especially the Toraja as a cough medicine. Empirically the community uses Miana leaves as a cough medicine by squeezing or pounding to take the juice to drink and boil with enough water. Testing the potential of Miana leaves as a cough medicine is done by proving Miana leaf extract can kill the bacteria that causes coughing. The use of Miana leaves as a cough medicine in South Sulawesi is empirically proven by previous studies. Studies conducted by practitioners of traditional healers in Tana Toraja District indicate that the Toraja people (South Sulawesi province) have used Miana leaves to treat all types of coughs such as asthma cough which is characterized by a state of shortness of breath, coughing up phlegm, dry cough (without phlegm) to cough accompanied by blood (Pakadang, et al., 2015). Research on the ethnopharmacology of medicinal plants as a cure for infectious diseases by Pakadang and Karim (2016) shows that Bugis and Makassarese in South Sulawesi also use Miana leaves as a medicine for phlegm cough and bloody cough.

Miana leaves used as research material were taken from Tana Toraja Regency. The test material is prepared by means of extracting the leaf juice using a juicer so that the juice and pulp are separated. Selection of cider as a test material to approach treatment by the people who use miana leaves as medicine. The results of the study turned out that Miana leaf extract gave a larger diameter inhibition zone for bacterial growth compared to cefadroxyl and amoxicillin antibiotics as comparative drugs. The potential of miana leaves as a drug is determined by the activity and content of the active substances contained in cells including flavonoids, tannins, polyphenols and saponins. Lumbessy (2013) has conducted a qualitative test of flavonoid content on iler, ketepeng, pearl grass, puzzle grass.
and gotu kola samples. As a result, all samples contained flavonoids with the highest total flavonoid content in ketepeng 26.86 mg / ml and iler 14.25 mg / ml. Other studies have shown that the active ingredient of flavonoids is not only potential as an antibacterial but can also be antioxidant.

The antibacterial mechanism of flavonoids in plants is to inhibit nucleic acid synthesis and cause damage to the permeability of bacterial cell walls, microsomes and lysosomes as a result of interactions between flavonoids and bacterial DNA, forming complex compounds with extracellular proteins and dissolving so that they can damage bacterial cell membranes and be followed by intracellular compounds. Other studies suggest the mechanism of flavonoids inhibits cell membrane function by disrupting the permeability of cell membranes and inhibiting enzyme bonds such as ATPase and phospholipase (Li et al, 2003; Cushnie at al, 2005; Nuria et al, 2009); Hendra et al, 2011). The antibacterial mechanism of phenol compounds in killing microorganisms is by denaturing cell proteins (Palczar, 1988). The mechanism of action of tannin as an antibacterial is protein agglutination. Tanin has antibacterial activity related to its ability to activate microbial cell adhesin, activate enzymes, and interfere with protein transport in the inner cell layer. Tanin also has a target on cell wall polypeptides so that the formation of cell walls becomes less perfect (Cowan, 1999; Akiyama, 2001; Nuria et al, 2009; Sari and Sari, 2011). The mechanism of action of saponins as antibacterial is that it can cause leakage of proteins and enzymes from within the cell. Saponins can be anti-bacterial because the surface active agents are similar to detergents, as a result saponins will reduce the surface tension of bacterial cell walls and damage membrane permeability. Saponins diffuse through the outer membrane and vulnerable cell walls and then bind to the cytoplasmic membrane so that it interferes with and reduces cell membrane stability. (Cavalieri et al, 2005; (Harborne, 2006; Madduluri et al., 2013). The mechanism of action of alkaloids as antibacterial is by disrupting the constituent components of peptidoglycan on bacterial cells so that the cell wall layer is not formed intact and causes cell death. Other mechanisms alkaloid components are known as DNA intercellators and inhibit bacterial cell topoisomerase enzymes (Karou et al., 2005; Evans, 2009; Darsana et al., 2011). The mechanism of steroids as antibacterial is related to lipid membranes and sensitivity to steroid components that cause liposome leakage. Steroids can interact with cell phospholipid membranes and cause fragile cell membranes and lysis (Ahmed, 2007; Madduluri et al, 2015).
Variations in the content of active substances from each type of sample or different family are influenced by many factors. One factor is where plants grow (Farming, 2017). Previous research has shown that the location where miana leaves grow affects the antioxidant activity of the extract produced. Miana leaf extracts planted in highland areas provide a better treatment potential compared to miana leaves grown in low-lying areas with higher air temperatures. The results showed that miana leaves planted in cool areas (cooler temperatures) such as Tana Toraja Regency and (Baumata Kota Kupang) provide better antioxidant potential than miana leaves grown in Makassar City (higher temperatures and pollution) (Pakadang and Hilaria, 2017).

The test bacteria used in this study were four types of bacteria that were isolated and identified from sputum cough culture (airway infection) in Ibnu Sina Hospital (Pakadang et al., 2017). The results of isolation from 10 sputum samples of non-tuberculous cough patients found 5 types of bacteria namely *Streptococcus pneumonia* (7 samples), *Klebsiella pneumonia* (1 sample), *Staphylococcus aureus* (2 samples), *Staphylococcus epidermidis* (3 samples) and *Enterobacter agglomerans* (1 sample) and fungus *Candida albicans* (1 sample).

In vitro testing with the bacterial growth incubation period was carried out more than 24 hours to prove the potential of bacteriocide miana leaf extract. The results obtained are Miana leaf extract can kill the test bacteria by maintaining a inhibition zone of more than 24 hours. This provides scientific evidence from empirical use in the community that miana leaves can treat cough because it can kill airway-causing bacteria with common manifestations of coughing. The potential of miana leaves as other antibacterials has also been proven by other researchers, antibacterial against *S. aureus*, *E. coli* and *P. aeruginosa* with leaf extract levels of 10-40% (Mpila et al. 2012). Yuningsih (2007) stated that the acetone extract of Miana leaf (which contains alkaloids and steroids) provided the largest inhibition zone for *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Kumala (2009) showed that Miana leaves with 1% extract concentration could inhibit *S. aureus* and *E. coli*.

According to the WHO report that every year 4 of the 13 million deaths in developing countries are caused by respiratory infections. The most common manifestation of airway infection is coughing. Coughing is a common manifestation of an airway infection. Coughing is a mechanism for the body’s immunity to clear antigens from the airways so that the higher the intensity of the cough is a strong indication of the occurrence of infection in the throat. Cough is classified as a productive cough.
and non-productive cough. Productive cough is a cough that produces phlegm. The results of the body’s defense mechanism against infectious agents will cause phlegm which contains a number of microorganisms including bacteria that cause infection, because phlegm in coughing indicates infection and inflammation of the respiratory tract (Ringel, 2009; Wilson, 2005).

CONCLUSION
Miana leaf extract has the potential as a cough medicine because it can kill the bacteria Streptococcus pneumonia, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia which is isolated from cough sputum. Miana leaf extract provides a better zone of bacterial growth inhibition than amoxicillin and cefradoxyl.

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